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The Influence of Visible Light on the Sulfhydryl Content of Yeast Cells
After Ionizing and Ultraviolet Irradiation

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REPORT NO. 72

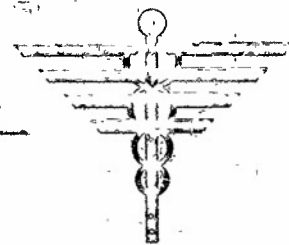
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THE INFLUENCE OF VISIBLE LIGHT ON THE SULFHYDRYL CONTENT OF YEAST CELLS AFTER IONIZING AND ULTRAVIOLET IRRADIATION*

*From Subtask 4, "Effect of Irradiation on Single Cell Organism",
of Project 6-59-08-013, "Effect of Irradiation".

MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY



REPORT NO. 72

THE INFLUENCE OF VISIBLE LIGHT ON THE
SULFHYDRYL CONTENT OF YEAST CELLS AFTER IONIZING
AND ULTRAVIOLET IRRADIATION*

by

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Fort Knox, Kentucky
15 December 1951

*From Subtask 4, "Effect of Irradiation on Single Cell Organism",
of Project 6-59-08-013, "Effect of Irradiation".

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AMRL Project No. 6-59-08-013-S-4

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ABSTRACT

THE INFLUENCE OF VISIBLE LIGHT ON THE SULFHYDRYL CONTENT OF YEAST CELLS AFTER IONIZING AND ULTRAVIOLET IRRADIATION

OBJECT

The investigations were undertaken to study changes in the thiol content of proteins in cells, as affected by ultraviolet and ionizing radiations. This report deals with the development of an amperometric method for the quantitative determination of sulfhydryl groups; its application for the titration of sulfhydryl groups in yeast cells; and some preliminary data on the sulfhydryl content of yeast cells as affected by x- and ultraviolet irradiation and subsequent irradiation with visible light.

RESULTS AND CONCLUSIONS

An amperometric method for the determination of sulfhydryl groups was developed, which permitted the detection of sulfhydryl compounds in amounts of 10^{-8} with a precision of at least $\pm 2\%$.

Evidence is presented that this method will permit the determination of sulfhydryl compounds within the yeast cells.

Yeast cultures irradiated with sufficient doses of ultraviolet light, or with soft x-rays, showed a decrease in sulfhydryl content as well as the number of viable cells. Subsequent irradiation of the ultraviolet irradiated cells with strong visible light led to a partial reversal-an increase in the sulfhydryl content as well as the number of viable cells.

Subsequent irradiation of the soft x-ray treated cells with visible light had no effect on the sulfhydryl content of the cells, but did lead to a slight increase in the viable cell count.

RECOMMENDATIONS

The effect of ionizing and ultraviolet irradiations and the subsequent effect of visible light on the sulfhydryl content and activity of isolated crystalline thiol enzymes, and thiol enzymes within the yeast cell should be studied.

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THE INFLUENCE OF VISIBLE LIGHT ON THE SULFHYDRYL CONTENT OF YEAST CELLS AFTER IONIZING AND ULTRAVIOLET IRRADIATION

I. INTRODUCTION

Compounds possessing sulfhydryl groups command a singular prominence in the living cell on account of their diversified and inseparable association with the mechanism of cell division (1), enzymic activity (2), and protein architecture (3).

The observation has been made repeatedly that cells rendered nonviable by ionizing or ultraviolet irradiations occasionally may be revived if stored under varying conditions after irradiation (4). The discovery of the phenomenon of photoreactivation (5), (the reviving effect of visible light on ultraviolet irradiated cells) has shed new light on the complexities of the irradiation phenomena, but the underlying mechanism of the observed effects is still unknown (6).

This report deals with the extent to which sulfhydryl groups within the cell are involved during ionizing and ultraviolet irradiation and the subsequent action of visible light.

II. METHODS AND PROCEDURES

A. Amperometric method for sulfhydryl groups.

The amperometric determination of mercaptans using the rotating platinum cathode (7) and its modifications (8) for the determination of thiol groups in proteins has recently been improved (9) by substituting a vibrating platinum electrode.

In the thiol determinations described below, the vibrating electrode was substituted for the rotating platinum electrode in the method of Benesch and Benesch (8). The vibrating electrode consisted of a platinum wire attached to the armature of an auto-radio vibrator connected to a 6-volt power supply. The mercury-mercuric iodide reference electrode consisted of a vessel, similar to that previously described (10), which contained about 0.6 ml of mercury on top of which was placed the electrolyte solution (4.2g of potassium iodide and 1.3g of mercuric iodide in sufficient saturated potassium chloride to make 100 ml). The salt bridge was filled with a 3% gel of agar containing 30% potassium chloride.

A light beam type of galvanometer with a sensitivity of 0.04 ua per division was connected to the electrodes. A variable resistor (100 ohms) was placed across the leads of the galvanometer. The occasional erratic response of this electrode was checked by day-to-day standardization of the silver nitrate.

The standardization of the silver nitrate was carried out by placing 1 ml of about 0.001 N thioglycollic acid, the purity of which had previously been determined, into a 100 ml beaker containing 29 ml of absolute ethanol and 1 ml of supporting electrolyte (30 g of ammonium nitrate, 125 ml of concentrated ammonia and sufficient distilled water to make 250 ml). The electrode was then started vibrating and the bridge opened. A few minutes were allowed for the galvanometer to come to rest at its zero point. Then small aliquots of 0.001 N AgNO_3 were added, waiting a few seconds for the galvanometer to register a steady state before taking the reading. The values obtained were plotted. The end point and calculations were carried out in the usual manner (11), using the formula:

$$\frac{N \text{ AgNO}_3 = N \text{ thioglycollic acid} \times \text{ml thioglycollic acid}}{\text{ml AgNO}_3}$$

The aliquots of yeast cell suspensions were titrated in the same manner as the silver nitrate standard except that a 20% methanol solution was used in place of the absolute ethanol. The per cent SH was then obtained by the formula:

$$\frac{\% \text{ SH} = \text{Vol AgNO}_3 \times N \text{ AgNO}_3 \times 0.033 \times 100}{\text{wt. of sample}}$$

B. Yeast Cultures

Saccharomyces cerevisiae (Meyers and Reis, Seagram #44*) was grown in malt extract broth (Difco) at 30°C for 18-24 hours in stationary cultures (250 ml medium in 1 liter Erlenmeyer flasks). The harvested cells were centrifuged and washed twice with 50 ml saline and suspended in saline to the desired volume. Irradiations were carried out immediately after these operations.

* We are grateful to Dr. M. C. Brockmann of Joseph Seagram and Sons, Louisville, Ky., who furnished us with transfers of this and other strains of yeasts for our studies.

C. Ionizing irradiations

A Picker-Waite x-ray diffraction unit, utilizing a Machlett-50-T thin beryllium (1 mm) window tube without additional filters was used to provide a source of soft x-rays. The tube was operated at 50 Kv and 18-ma. The target specimen was placed 10 cm from the tube. The yeast saline suspension was irradiated in a depth of 1 mm in stainless steel dishes, 36 mm diameter. The x-ray dosage was calculated to be about 20,000 r/min. **

D. Ultraviolet irradiations

For the ultraviolet irradiations a 15 W G. E. germicidal lamp, intensity about $50 \text{ ergs} \times \text{sec}^{-1} \times \text{mm}^{-2}$, was placed 60 cm from the yeast saline suspension (depth of 1 mm). The radiation of this lamp is practically monochromatic, 95% of the light source being at $2,537 \text{ \AA}$.

E. Visible light irradiations

Irradiation with visible light was accomplished by the use of a G. E. A-H4 mercury lamp (100 W) with a fixed reflector but no condenser. It was placed 20 cm from the sample to be irradiated. A liquid filter consisting of a 1 cm layer of 5% CuSO_4 was used to remove most of the infrared.

F. Cell Counts

The cell counts were made by appropriately diluting the yeast suspension with saline and streaking on malt agar plates. The organisms were shaken mechanically (Kahn shaker) prior to diluting to avoid clumping. Final cell counts were made after 48 hours incubation of the malt agar plates.

The dry weight of the yeast was determined by drying 2 ml aliquots of the yeast saline suspension in shallow pans at 90°C for two hours. The dry weights were corrected for the NaCl present.

** The x-irradiations as well as calculations of roentgens were carried out by the radiobiology department.

III. RESULTS

A. Determination of thiol groups in the intact and broken yeast cell

In order to ascertain whether amperometric titration would permit a quantitative determination of the thiol groups in the intact yeast cell, a comparison of the SH content of the intact and disrupted yeast cells was carried out. The disruption of the yeast cells was accomplished by the use of a stainless steel percussion apparatus (12) which was cooled with a dry ice-acetone mixture. This method permitted practically complete disintegration of the cells in a minimum of time under very mild conditions. The efficiency of the percussion method was evaluated as percent kill by making viable counts before and after treatment and by gross microscopic examination.

A comparison of the two experiments (Table 1) showed that identical amounts of thiol groups were found in the intact yeast cells, and the disintegrated cells. It has been shown previously (9) that thiol groups can be titrated in the intact protein molecule by a similar amperometric method.

B. Effect of ultraviolet irradiation on yeast cells

The source of ultraviolet light was a G. E. germicidal lamp, 60 cm from the target. Yeast cells suspended in saline were irradiated in steel planchets. Plotting the logarithm of the surviving cells versus increasing increments of ultraviolet irradiation resulted in a straight line relationship. (Figure 1A)

A washed yeast suspension in saline was placed in stainless steel dishes at a depth of 1 mm. Aliquots of the yeast suspension were irradiated for 2-15 minutes with ultraviolet light and the SH content and viable cell count determined. Another aliquot of the cell suspension was treated identically, but immediately after the ultraviolet irradiation was irradiated with visible light. Suitable controls were carried out under identical conditions. Unwanted photoreactivation of the yeast cells from light needed for working was forestalled by keeping the cells in the dark and exposing them only to weak yellow light from a G. E. 25 A/OAO lamp during the handling.

The results of one of these experiments is shown in Table 2. Exposure to ultraviolet light resulted in a decrease of SH groups and surviving cells. Subsequent illumination with strong visible light resulted in a small, but definite increase in SH groups and a

considerable increase in the viable cell count compared with cells which were treated with ultraviolet light only.

C. Effect of ionizing radiations on yeast cells

A yeast cell suspension which had received a dose of 100,000 r. was incubated in malt extract broth and the progress of growth observed under the microscope. During the first 1-2 hours the cells appeared normal, except for an occasional cell that was small and shrunken. A large number of cells formed doublets with a peculiar dumbbell shape. During the next 2-4 hours there was a gradual increase in cell size, most of the cells congregating in clusters of 2-12. The maximum cell size was apparently reached after 6 hours of incubation.

Prior to the determination of sulfhydryl groups in x-irradiated yeast cells, a desirable x-ray dosage range was determined by irradiating washed yeast suspensions in saline with varying doses of x-rays (Fig. 1B).

In order to determine the changes in the sulfhydryl content of yeast cells after ionizing irradiation, a washed suspension of yeast cells in saline was divided into various aliquots and treated as follows: One portion was x-irradiated with 100,000 r, another portion after identical x-irradiation treatment was exposed immediately to strong visible light for 30 minutes under conditions outlined above, while a control portion was kept under identical conditions of temperature and storage. In all cases the sulfhydryl content and viable cell count was determined immediately at the end of the experiment. In order to avoid possible undesired side reactions, the yeast cells were shielded against light, and the handling after exposure to irradiation was carried out by illuminating the laboratory with weak yellow light only (G. E. 25 A/OAO lamp).

The results of a typical experiment are given in Table 2. A considerable decrease in the SH content occurred after x-irradiation together with a decrease in the viable cell count as compared with the control. Illumination with strong visible light for 30 min. after x-irradiation did not effect the SH content. However there was a slight increase in the viable cell count.

IV. DISCUSSION

Whether or not this slight increase was caused by a catalytic action of traces of heavy metal (stainless steel containers were used in the x-irradiation experiments) remains to be determined. In this connection it should be pointed out that a slight amount of photoreactivation after x-irradiation was also obtained with T2 bacteriophage (13).

The consistent increase in the thiol content of ultraviolet exposed cells following visible light irradiation may be significant. The formation of a light labile toxin under the influence of ultraviolet irradiation and its destruction by visible light has been postulated (6). Changes in the SS-SH ratio within the cell following ultraviolet and light irradiation may well be associated with the mechanism of photoactivation.

V. CONCLUSIONS

An amperometric method for the determination of sulfhydryl groups was developed, which permitted the detection of sulfhydryl compounds in amounts of 195 with a precision of at least $\pm 2\%$.

Evidence is presented that this method will permit the determination of sulfhydryl compounds within the yeast cells.

Yeast cultures irradiated with sufficient doses of ultraviolet light, or with soft x-rays, showed a decrease in sulfhydryl content as well as the number of viable cells. Subsequent irradiation of the ultraviolet irradiated cells with strong visible light led to a partial reversal-an increase in the sulfhydryl content as well as the number of viable cells.

Subsequent irradiation of the soft x-ray treated cells with visible light had no effect on the sulfhydryl content of the cells, but did lead to a slight increase in the viable cell count.

VI. RECOMMENDATIONS

The effects of ionizing and ultraviolet irradiations and the subsequent effect of visible light on the sulfhydryl content and activity of isolated crystalline thiol enzymes, and thiol enzymes within the yeast cell should be studied.

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TABLE 1

COMPARISON IN THE SULFHYDRYL CONTENT OF INTACT
AND DISINTEGRATED YEAST CELLS AS DETERMINED BY
AMPEROMETRIC TITRATION

Treatment	Number of Viable Cells (1)	% SH	Average SH%	%Destruction of Cells
Intact Cells	1.25×10^9	0.378 0.368	0.373	
Disintegrated Cells	2.34×10^6	0.373 0.383	0.378	99.8
Intact Cells	4.80×10^9	0.265 0.272	0.269	
Disintegrated Cells	6.24×10^6	0.260 0.255	0.258	99.9

(1) Average of 2 determinations

TABLE 2

THE EFFECT OF ULTRAVIOLET AND IONIZING IRRADIATION
AND SUBSEQUENT IRRADIATION WITH VISIBLE LIGHT ON THE
SH CONTENT AND VIABLE CELL COUNT OF YEAST CELLS

	Dosage	Illumination with Visible Light (2)	% SH (3)	Viable Cell Count (4)
X-ray $r \times 10^4$	Ultraviolet Light (min) (1)			
-	-	-	0.209	2.49×10^9
10	-	-	0.119	4.32×10^8
10	-	30	0.118	6.43×10^8
-	-	-	0.212	5.73×10^9
-	3	-	0.115	8.40×10^8
-	3	30	0.152	1.46×10^9

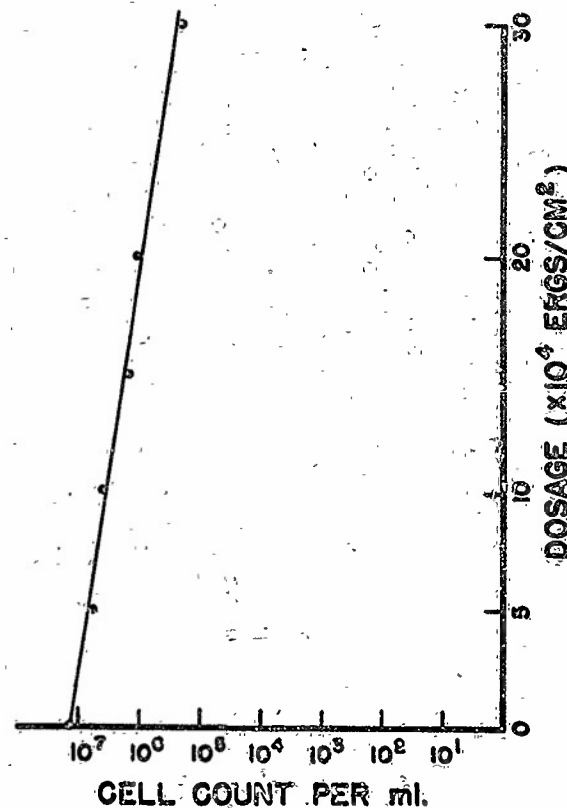
(1) 15 watt G. E. germicidal lamp, 60 cm target distance

(2) G. E. A-H4 mercury lamp (100 W) 20 cm distance with 1 cm 5%
 CuSO_4 filter interposed.

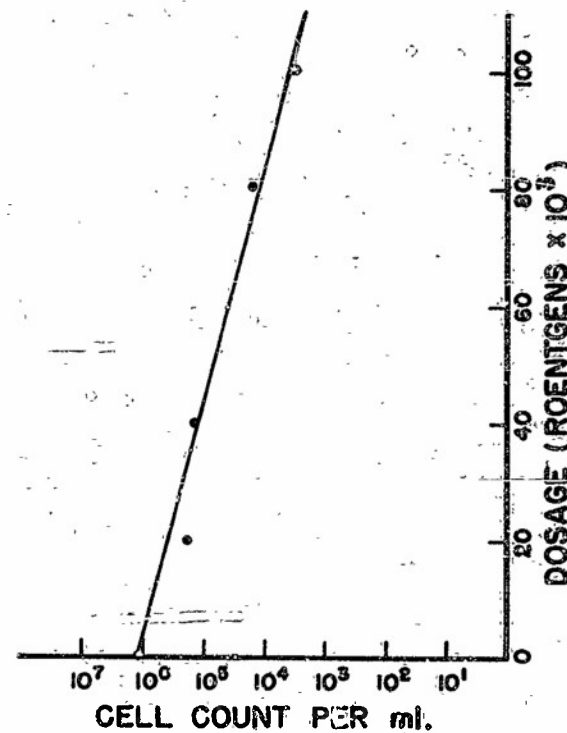
(3) Average of 2 determinations.

(4) Average of 3 counts.

A
EFFECT OF ULTRA-VIOLET LIGHT ON A
YEAST CELL SUSPENSION IN SALINE.



B
EFFECT OF X-RAYS ON A YEAST
CELL SUSPENSION IN SALINE.



**FIG. 1 THE EFFECT OF VARIOUS DOSAGES OF
ULTRA-VIOLET LIGHT AND X-RAYS ON
YEAST SUSPENSIONS IN SALINE.**